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Biological Treasures

Tiny seeds, a fuzzy mold, a 30-foot-long tapeworm, an imported wasp—these are random items from our national biological treasures. They are in several permanent and priceless collections maintained by ARS as working tools for scientists of many disciplines. But the value of these collections transcends the merely utilitarian for they are also links in the chain of life that mankind itself requires for survival.

The National Seed Laboratory at Fort Collins, Colo., for example, preserves valuable germ plasm to aid the world's plant breeders in improving old crops. This facility also tries to save from extinction seeds of wild and primitive plants that are endowed with irreplaceable genetic qualities such as disease resistance or high protein content (see page 8).

Similarly, the ARS Culture Collection at Peoria, Ill., is a living reserve of some 17,000 species of bacteria, molds, and yeasts. Scientists have harnessed the energy of microbes in this collection to produce penicillin, vitamins B_2 and B_{12}, dextran, and soybean foods.

Two important collections are maintained at the Agricultural Research Center. The Animal Parasite Collection, along with its Index-Catalogue of Medical and Veterinary Zoology, forms one of the world's most complete information centers on parasites affecting animals and man. During World War II, for example, it provided information on parasites likely to be encountered by farflung Allied forces. The National Fungus Collections, the world's largest, also extends thorough reference services. In the last 10 years, over 26,000 specimens have been loaned to institutions and individuals to study or to confirm identifications. Since the collections record all described fungi and contain all the major disease-causing fungi, they constitute a tangible history of plant diseases. This background enables scientists to quickly determine if a disease outbreak is due to a known species or to a new one.

Another important collection embraces 17 million insect species in the Smithsonian National Museum of Natural History, under the custody of ARS and Smithsonian entomologists. It provides identification services as well as information on distribution and life histories to entomologists who track down the natural enemies of insect pests, an approach that poses no hazard to other life or the environment.

These collections serve many immediate needs. Some of them also help to improve our understanding of the irreplaceables in the web of life—a vital concern because the evolutionary force that put us on this planet still goes on.

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COVER: The nutritional value of U.S. corn hybrids may be enriched through crossbreeding with a stone-age corn from South America. Such primitive plants, often tossed aside or carelessly destroyed, are an important source of valuable genetic material bypassed in developing modern crops. See related articles on pages 3 and 8 (PN–1992).

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Genes from primitive Coroico corn at left may be used to improve the nutritional value of U.S. commercial hybrids such as the other ear (PN–1980).

Retrieving genes for SPACE-AGE CORN

GENES HIDDEN in corn cultivated since the stone age were salvaged for space-age hybrids when ARS scientists discovered that the primitive kernels have an extra thick aleurone layer.

Aleurone, the site of B-vitamins and high-quality protein just under the kernel hull, is two to five cells thicker in primitive Coroico corn than in U.S. commercial hybrids. Thus Coroico, which grows on the eastern slopes of the Andes Mountains, might serve as parent stock to improve the nutritional value of U.S. hybrids.

This discovery demonstrates the recovery of genetic material bypassed in developing modern corn. Most ancestors of U.S. hybrids trace through open-pollinated corns to two main lines—dent corns, probably originating in

Mexico, and flint corns, similar to some in Guatemala.

The thick aleurone layers were found in Coroico by chemist and microscopist Michael J. Wolf and botanist Uheng Khoo at the Northern marketing and nutrition research laboratory, Peoria, Ill.

Fifteen samples of Coroico aleurone averaged 3.7 cell layers and 3.4 percent of whole kernel weight. Aleurone is one cell thick and makes up less than 2 percent of the kernel in other corn. Weight and number of layers are not proportional because cell sizes vary more in multiple than in single layers.

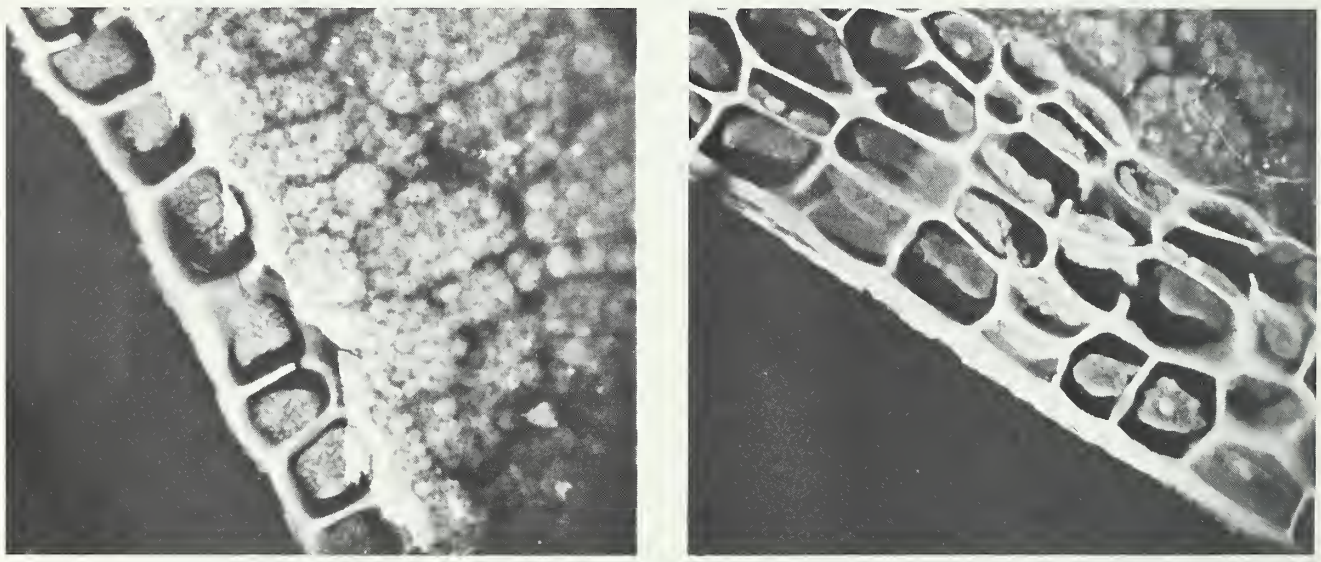
Besides making up more of the kernel, Coroico aleurone contains a higher proportion of protein than dent aleurone. Crude protein in Coroico aleurone varied from 35 to 38 percent in Northern laboratory analyses. The aleurone in dent is 20- to 22-percent protein.

Moreover, Coroico corn contains more of the essential amino acids lysine, arginine, methionine, and threonine. The scientists did not analyze Coroico for vitamins, but cereal aleurone is recognized for its concentration of B-vitamins, especially niacin.

Dr. Wolf first found multiple layers of aleurone in corn bred by Marcus S. Zuber, ARS plant breeder at the University of Missouri. Dr. Zuber had crossed U.S. corn with Peru 442, a South American corn of mixed ancestry. With the help of Dr. Hugh C. Cutler, botanist at the Missouri Botanical Garden, Dr. Wolf traced the multicell layers through Peru 442 to Coroico. Dr. Cutler found the corn in Bolivia and described it more than 25 years ago.

Further crosses of Coroico or Peru 442 with U.S. inbred corns suggest that partially dominant genes control multiple layering in aleurone. Offspring of such crosses have blends of the parents' partially dominant traits. □

These photomicrographs contrast the one layer of cells in the aleurone of a U.S. hybrid with the four to five layers in Coroico aleurone (PN–1981, PN–1982).



Fungicide controls twig dieback

A SYSTEMIC FUNGICIDE may provide an answer to twig dieback in Robinson hybrid tangerines.

Twig dieback can reduce fruit yield of older trees by 1 to 10 percent. Young trees may die or require subsequent pruning. Yield reductions cause high income losses to Robinson tangerine growers because the fruit, ready for market very early, may bring double the price of the later main crop.

When a tree is attacked, usually in early summer, the twigs droop, turn brown, and die. In later stages the disease kills branches. Observation of the Robinson and other tangerine hybrids indicates that susceptibility to twig dieback may be inherited, but the exact cause of the disease is unknown.

The systemic fungicide methyl-1-(butylcarbamoyl) - 2 - benzimidazolecarbamate (benomyl) had previously proved effective in controlling twig dieback of citrus. It was first used under field conditions with Robinson tangerines by ARS geneticist C. Jack Hearn, Orlando, Fla.

He applied a foliage spray (5 ounces of active ingredient per 100 gallons) in March when the first spring flush of growth appeared and in June to coincide with the second flush of growth. Only 8 percent of the trees receiving two treatments showed dieback, in contrast with 39 percent of the untreated trees.

Further research is needed to insure the safety of the fungicide treatment as well as to determine the most economical and effective procedures under commercial conditions.

Benomyl is not registered for use on citrus crops. Before a pesticide can be released to the public, it must undergo stringent tests by its manufacturer, who then submits test data and the product to the Federal Government for evaluation and registration. □

FOUND:

Chemosterilant for boll weevils

BUSULFAN, one of hundreds of chemical compounds tested, has been found to make male boll weevils sexually sterile with little or no other damage to the weevil.

The achievement represents a major step toward integrating several control procedures for eradicating this cotton pest. In the next 2 years, sterilized male weevils will be released in a 25,000-acre test area of Mississippi, Alabama, and Louisiana to determine the feasibility of eradication.

Scientists theorize that busulfan, like other chemosterilants and radiation, breaks the boll weevils' chromosomes in the sperm, thereby producing mutations which prevent development of the embryo after union of the sperm and the egg.

One of the major advantages of busulfan is its permanent effect on males; other tested compounds induce only temporary sterility. Busulfan also results in little damage to the digestive system—a cause of premature death in weevils exposed to irradiation.

Credit for developing busulfan as a sterilant is shared by a team of scientists at four ARS laboratories involving chemist Alexej B. Borkovec and entomologists Waldemar L. Klassen, Hollis M. Flint, Norman W. Earle, Theodore B. Davich, Norman Mitlan, and Jack W. Haynes, and Southern Research Institute entomologists Dan Grizwold and David G. McHaffey.

Dr. Klassen and Dr. Earle worked out a treatment system for busulfan to combine as many desired effects as possible with a minimum of adverse effects. The objective: Virtually complete sterility without drastically shortening the weevils' lives or impairing their competitiveness with untreated weevils. The scientists got best results by feeding adult weevils for 6 days on an artificial diet containing 0.1 percent busulfan.

In a longevity test, only four of 161 male weevils that survived for 3 weeks developed viable sperm after treatment with busulfan. This represents a 98-percent success rate for inducing permanent sterility in male boll weevils.

Boll weevil works its way across a cotton boll, puncturing and chewing with its long, slender bill (BN-24072).

Sterilized males also proved competitive with normal males in related tests. One measure of the sterilized weevils' performance concerned the breeding characteristics they share with many insect species: Boll weevils mate more than once, and the sperm is stored in the females' bodies after mating until fertilization. Sperm from normal weevils was displaced with the genetically defective sperm from sterilized weevils during the major part of the females' lifespan.

Sterilized males also meet the competitive conditions of another biological peculiarity of boll weevils, production of the insects' natural sex attractant emitted by the males. Sterilized males attracted as many boll weevils as did normal males.

Busulfan is an experimental material and has not been registered for commercial use. Before this compound can be released to the public, it must undergo stringent tests by its manufacturer, who then submits test data to the Federal government for evaluation and registration. □

Mr. Vicente-Chandler, third from left, with government and university officials examines netting spread under coffee bushes (PN–1983).

Puerto Rico

CATCHING the COFFEE

A SYSTEM of harvesting coffee with low-cost plastic netting could revolutionize the industry in Puerto Rico and throughout the coffee-growing world.

This ARS-Puerto Rico Agricultural Experiment Station technique promises to raise the wages of coffee plantation workers, the net income of coffee farmers, and the harvested yield of coffee, while cutting the number of workers in a labor-short area by at least 75 percent.

Another benefit is the elimination of tree pruning to facilitate reaching the top berries. That also reduces labor since the shade of fuller trees restricts weed growth.

The plastic netting (20 by 11 feet) is simply spread on the ground between the rows of trees to catch mature berries as they fall, then is gathered by two men about every 6 weeks during the 4-month harvest season. After the nets are emptied at a central collecting point, they are immediately spread out again to catch more of the maturing berries.

Coffee in Puerto Rico, as in much of the world, is harvested by going over the plantations several times during the picking season, collecting only the ripe berries, which are dropped into baskets tied to the picker's waist. The pickers currently earn $1 per 5 gallons of berries, or about 20 cents per pound of market coffee. This amounts to about 50 cents an hour, but picking costs still approach $400 per acre yearly.

The new system of harvesting involves only 20 man-days on a 15-acre plantation compared to 66 man-days the old way. It also curbs heavy losses of high-yield plantations (2,000 pounds or more of market coffee per acre) from berries falling to the ground during the picking season, losses averaging $600 per acre yearly.

This combination of savings promises

to raise the net income of coffee growers from $237 to $596 per acre yearly, while doubling the wages of plantation workers.

Moreover, labor is much more evenly distributed over the year. A 15-acre plantation would require about four laborers during the picking season and two during the remainder of the year. Currently, such a plantation needs an average of 15 laborers during the picking season (as many as 20 during peak periods) and only two the rest of the year. Under the new system, coffee can be harvested when convenient—during clear weather, when labor is available, and when machinery is operating efficiently. And coffee trees escape damages associated with the present hand-picking system.

The researchers also found that when the net is collected at short intervals, berries predominate. When collected at 6-week intervals, 75 percent or more of the coffee is in the form of beans (parchment coffee), the pulp having decomposed naturally, without heat and without damage to the bean.

At the collection area, the material in the nets is run through a tunnel blower that removes most leaves and twigs. Then the beans are washed in a conventional horizontal coffee-washing machine which removes all the fine trash and coffee pulp of the few unfermented berries.

ARS researchers and the Puerto Rico Agricultural Experiment Station are continuing the study to perfect the cleaning process after the coffee is harvested.

The same ARS researchers—soil scientist Jose Vincente-Chandler, soil scientist Fernando Abruna, and technician Servando Silva—developed the complete package of technical practices for high-yielding plantations and the new harvesting procedures. Puerto Rican subsidies amounting to over $500 per acre to farmers using these practices will help defray costs of net stallation and maintenance. □

magnets detect insects

MAGNETS can't attract insects, but they may help detect hidden infestations in stored grains through a principle known as nuclear magnetic resonance.

Various methods for detecting hidden stored-grain insect infestations have been developed or proposed, including chemical, electrical, X-ray, and aural or sound. Each has certain major disadvantages.

ARS physicist M. Wayne Street at Savannah, Ga., suggests nuclear magnetic resonance (NMR), as a promising answer to insect detection problems. NMR is a well-known tool in physics that has also been used to determine moisture in wheat, flour, and dough, and to analyze soybean oil content.

Protons, the positively charged particles in an atomic nucleus, aline themselves in straight patterns when influenced externally by a strong direct-current magnetic field. If an alternating-current field of a very specific frequency, called the resonance frequency, is superimposed on the magnetic field, the protons absorb energy then "flip" or change orientation. This energy absorption is detected by the NMR spectrometer used to study the structure and molecular environment of the affected protons.

Believing that insects within the grain kernels would produce an NMR response of a magnitude sufficient to reveal their presence, Mr. Street ran a study using hard red winter wheat at three moisture values commonly encountered under grain storage conditions.

Since moisture content affects NMR responses, baseline response curves were charted for the test wheat at the three moisture levels before three tests were run to detect rice weevils and red flour beetles (two good representatives of stored-grain pests).

For the first test, Mr. Street scanned sample tubes containing 20 adult and larval test insects and obtained strong NMR responses. Next, he interspersed adults and larvae in sample tubes among clean, uninfested wheat grains, and they, too, gave good NMR signals.

The final objective of Mr. Street's study was to detect immature insects within wheat grains. He scattered several hundred wheat grains in one layer over an adhesive-coated plastic sheet, then employed X-rays to identify infested and uninfested grains and separate them for individual scanning. The NMR response obtained from the infested grains was distinguishable from that of noninfested grains. The NMR spectrometer used by Mr. Street was elaborate and expensive, but a similar less expensive instrument should be adequate for field use.

The exact nature of the insect-induced NMR response is unknown. Moisture and protein within the insect's body, as well as its fecal matter, are suspected contributors to this response.

Regardless of origin, NMR responses to insects dwelling inside kernels show promise as a future means for stored-product insect detection. □

Above: Louis N. Bass, head of the laboratory, discusses seed storage with former laboratory head Edwin James (center) and technician Dorris C. Clark (right) in front of the tri-level facility. The laboratory houses over 80,000 seed samples with a maximum capacity of 300,000 (1270A1163–22).

Right: Dr. Bass carries test tray out of the walk-in germination chamber. Seeds are tested for germination before storage and every 5 years thereafter (1270A1176–9).

Technician Judy Lewis employs vacuum counter to plant seeds on blue-grey germination paper. Technician William Wise plants larger seeds using a counting board and special towelling. They will place seeds in germination chambers (background) or in walk-in chambers which are designed to provide optimum germination environment for each kind of seed (1270A1167–7).

Laboratory aide David Cantonwine blows foreign material from grass seed before placing the seed into the metal storage cans (1270A1178–18).

Mr. Clark checks a 30-day chart which keeps a continuous record of temperature, which is maintained at 40° F. with relative humidity of 32 percent. Laboratory staff enter rooms only when necessary (1164–6).

Seeds for survival

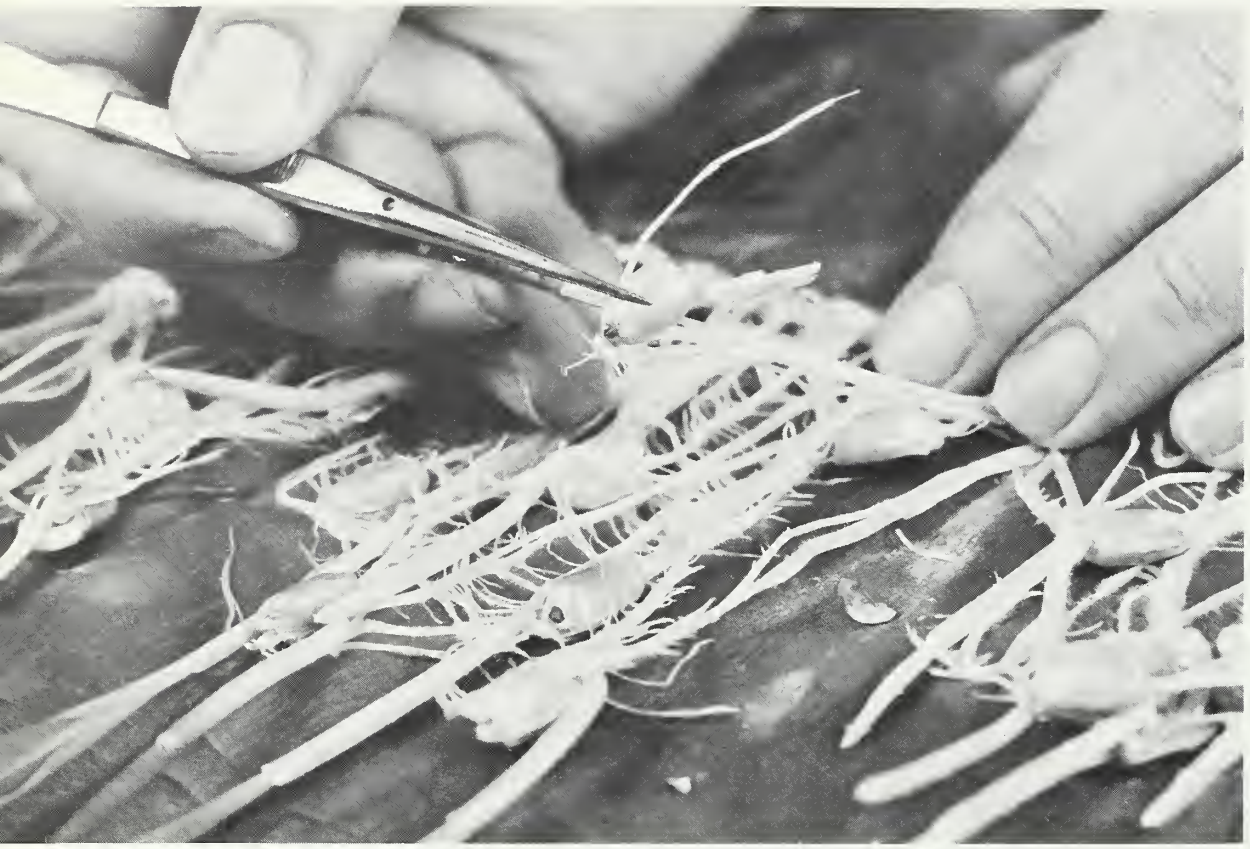
THE INTRODUCTION of seeds and plants from throughout the world has literally created American agriculture.

From early Colonial times, settlers brought seeds and cuttings of familiar and useful plants from their homelands. Today agricultural explorers search the world for new crops or for old forms of familiar crops with special characteristics needed by modern agriculture.

With very few exceptions, the crops now grown in the United States originated elsewhere—potatoes and tomatoes in South America; wheat and oats in the Near East; soybeans in China; rice in southeast Asia; cucumbers in India; cotton in Mexico and Central America; citrus in Malaysia—to cite only a few of our imported crops.

Since 1898, when USDA started numbering plant introductions, up to 1970, 350,000 kinds of plants were introduced into this country. But much of this valuable and often irreplacable germ plasm has been lost. Of the clovers introduced into the United States during the past 60 or 70 years, for example, only 2 percent are available today. Over 65 percent of the introduced oats have been lost, and 90 percent of the soybeans.

To prevent such future losses, ARS opened the National Seed Storage Laboratory in Ft. Collins, Colo., in 1958. Today, the laboratory stores some 80,000 different kinds of seeds representing many agricultural crops.



Mr. Clark evaluates corn germination test. Seeds are left in germination chambers for a specific number of days—7 for corn—then the number of normal and abnormal seedlings and dead seeds are counted. Abnormal seedlings are those which do not have the necessary parts to produce a plant. In right photo, seedlings in foreground are cotton and, middle row, peanuts (1270A1181–19, 1270A1181–16).

For easy retrieval, each kind of seed is catalogued on a punch card according to its special characteristics. Seeds are kept in pint cans in 11 cold storage rooms which are maintained at a temperature of 40° F. and 32 percent relative humidity.

Every 5 years scientists test for germination ability. If deterioration has occurred, contracts are made with seed-producing agencies to replenish the stock.

Anyone may submit seed of known value that is qualified for storage. But once stored, the seed becomes the property of the Federal Government and is available to all research scientists in the United States and, with special permission, to scientists abroad. However, there is no central repository for plant material which does not breed true from seed, such as fruits and some other crop plants that are propagated by vegetative means.

The production of food is a constantly shifting scene. Changes in agricultural practices or new forms of diseases demand crop plants with new characteristics. A new race of wheat rust appears—a rust-resistant source must be found and bred into our high-yielding wheats. Mechanized harvesting of tomatoes becomes necessary—strains must be found and bred that can survive the rigors of mechanical harvesting.

Moreover, through man's selection and breeding of agricultural plants over thousands of years, certain desirable qualities were often unwittingly sacrificed—sometimes the ability to resist a certain disease. The ancestors of today's crops often are the only sources of these qualities. And these primitive crop plants become scarcer each day, especially in developing countries as farmers adopt commercial varieties. And everywhere agriculture, development, and urbanization uproot primitive plant communities.

Today, with agriculture's growing emphasis on breeding disease and insect resistance into our food crops and increasing yields to feed a hungry world, plant breeders require large stocks of germ plasm to find a particular plant characteristic needed at any particular time.

If the proper plant material is not available, an expedition may be sent out to collect the needed plants or seeds. For example, ARS has sent expeditions into southern Mexico to collect certain cottons (called dooryard cottons) that grow around the huts of isolated Mexican farmers. These older, more primitive-type cottons are the best known source for insect, nematode, and bollworm resistance.

Besides serving as a "seed bank," the Ft. Collins laboratory is also incorporating research on various long-term storage problems of seeds, such as temperature and humidity, packaging, and germination requirements.

In a sophisticated agricultural nation such as ours, it is startling to realize that the future of many of our food crops could very well rest in small cans of seeds stored in a building nestled beside the foothills of the Rockies. □

Monitoring cleanliness in

MILK PROCESSING SYSTEMS

HOW CLEAN is clean in dairy processing plants? Part of the answer comes from an electronic device that monitors the removal rate of milk residue during the rinse phase of the cleaning cycle.

When milk passes through processing pipes, it deposits residues of fat and other material conducive to bacterial growth. This material must be removed. With the advent of cleaned-in-place (CIP) systems, which permit the cleaning of equipment without taking it apart (AGR. RES., Dec. 1967, p. 4), the removal rate of milk residue should be continuously measured to assure maximum cleanliness.

ARS agricultural engineers Maynard E. Anderson and James R. Fischer, working with microbiologist Robert T. Marshall and agricultural engineers Donald B. Brooker and Eliseo L. Ruiz of the Missouri Agricultural Experiment Station, Columbia, have successfully measured the removal rate by monitoring the water-milk mixture's electrical resistance change during rinsing.

Use of electrical resistance change is based on the theory that milk deposits removed from equipment surfaces contain certain ionizable substances that can be detected by electrical resistance changes.

To test this theory, the researchers evaluated a cleaning operation with the electrical resistance method, then compared the results with an evaluation made by a spectrophotometric analysis, a method of measuring residual calcium remaining in the pipes after cleaning.

The electrical system tested contained a Wheatstone bridge whose conductivity cell's electrodes sensed changes in the resistance of the water-milk mixture and registered these changes in the output voltage.

The test piping system included an insulated 57-gallon reservoir, a variable speed pumping unit with centrifugal pump and variable speed controller, and a turbine flow meter accurate to 0.5 percent.

Rinse water flow rate and temperature were set at 15, 30, and 45 gallons per minute and 95° and 125° F. Pipes were flushed under various combinations of these settings.

The electrical resistance technique accurately determined the point of complete removal of ionizable soil from the equipment. Under controlled conditions, the amount of soil removed could be determined. This testing method also complements the spectrophotometric calcium analysis, since electronic monitoring shows how much residue is removed from the pipes.

The test indicated that the various flow rates and temperatures provided different removal rates. Any discrepancy in the amounts of residue removed under the different flow rates was attributed to extent of milk residue ionization in the rinse solution. □

Packaged for sale, milk moves down the line in a processing plant (PN–1984).

Easy-to-read
ANGLE BRANDS

TRYING TO FIND an angle may be the right approach when it comes to designing highly visible livestock brands.

ARS veterinary scientist R. Keith Farrell, Pullman, Wash., designed a system based on angles which is unalterable and easily read from a distance.

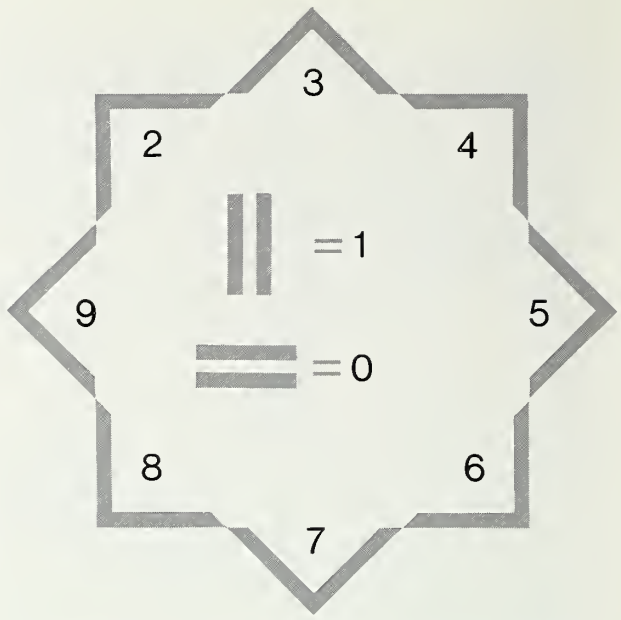
The system is based on two concentric squares. In the basic square, numbering starts in the upper left corner with two, and continues clockwise with four, six, and eight. The odd numbers are obtained by rotating the basic square an eighth turn to the right so the corners fill in the gaps between the even numbers. Numbering begins at the top with three and runs clockwise to five, seven, and nine.

Various combinations of angles, with vertical and horizontal parallel lines for one and zero, respectively, are used to form symbols. Position of the angles relative to the concentric squares indicates how the symbols are read.

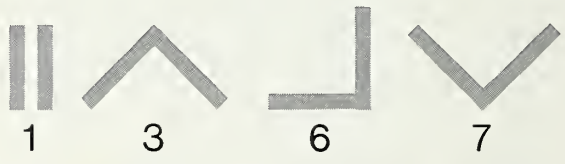
Animals were marked with this system by tattoo, freeze brand, and laser.

In recognition trials, the angles were more easily read than numbers even though people are more familiar with numbers. For example, at 40 feet only 59 percent of the numbers were recognized compared to 90 percent of the angle symbols.

Dr. Farrell has done previous work with freeze branding, an international identification system, and laser beams for marking (AGR, RES., June 1966, p. 8; Feb. 1971, p. 10; April 1971, p. 15). All this work relates to animal identification for breeding, disease control programs, or legal reasons. □



Master "clock face," formed by two concentric squares, shows the angles and the numbers they represent (PN–1985).



Researchers read this brand, number 1367, by matching the angles against the clock face (PN–1986).

Aflatoxin affects disease resistance

AFLATOXIN, a fungus-produced toxin in moldy animal feed, impairs protein synthesis, a finding that led ARS scientists to investigate its effects on acquired disease resistance.

Impaired protein synthesis may affect the production of specific antibodies to diseases or the levels of nonspecific substances such as interferon, which impart resistance to a wide range of viruses.

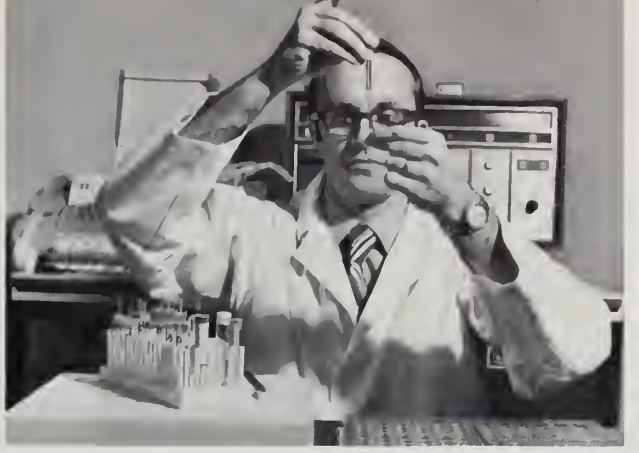
Working with turkey poults, the ARS veterinary scientists immunized the birds with inactivated bacterial or viral vaccines before, during, or after a 3-week aflatoxin feeding period.

Immunity was then challenged by inoculation with virulent bacteria or virus organisms given 3 weeks after vaccination, 3 weeks after the start of aflatoxin feeding, or after a 3-week "recovery period" from aflatoxin.

Results indicated that aflatoxin consumption reduced acquired resistance to bacterial challenge when vaccine was given during or before aflatoxin. There was no reduction when vaccine was given after aflatoxin feeding. The reasons for these results are still under investigation.

Viral resistance was not diminished from any combination of aflatoxin feeding and vaccination.

The research was done by ARS veterinary scientists Allan C. Pier, Kenneth L. Heddleston, and William A. Boney at the National Animal Disease Laboratory, Ames, Iowa, with cooperation from Phillip D. Lukert of the University of Georgia. □



Part of fingerprinting process. ***Left:*** *Chemist Clarence A. Knutson weighs biopolymer samples (PN–1987).* ***Top right:*** *Chemist Paul A. Sandford transfers hydrolyzed polymer for radioactive tagging and separation by paper chromatography (PN–1988).* ***Bottom right:*** *Tagged chromatograms are fed into liquid scintillation counter for a mathematical print-out of quantitative analysis (PN–1989).*

'Fingerprinting' rare compounds

RARE COMPOUNDS isolated by Indian scientists from exotic plant gums are helping ARS chemists select new types of polysaccharides suitable for practical uses in industry and medicine.

The 5-year Indian project, sponsored by ARS under a Public Law 480 grant, was conducted at the National Sugar Institute, Kanpur, under the direction of Dr. S. Mukherjee.

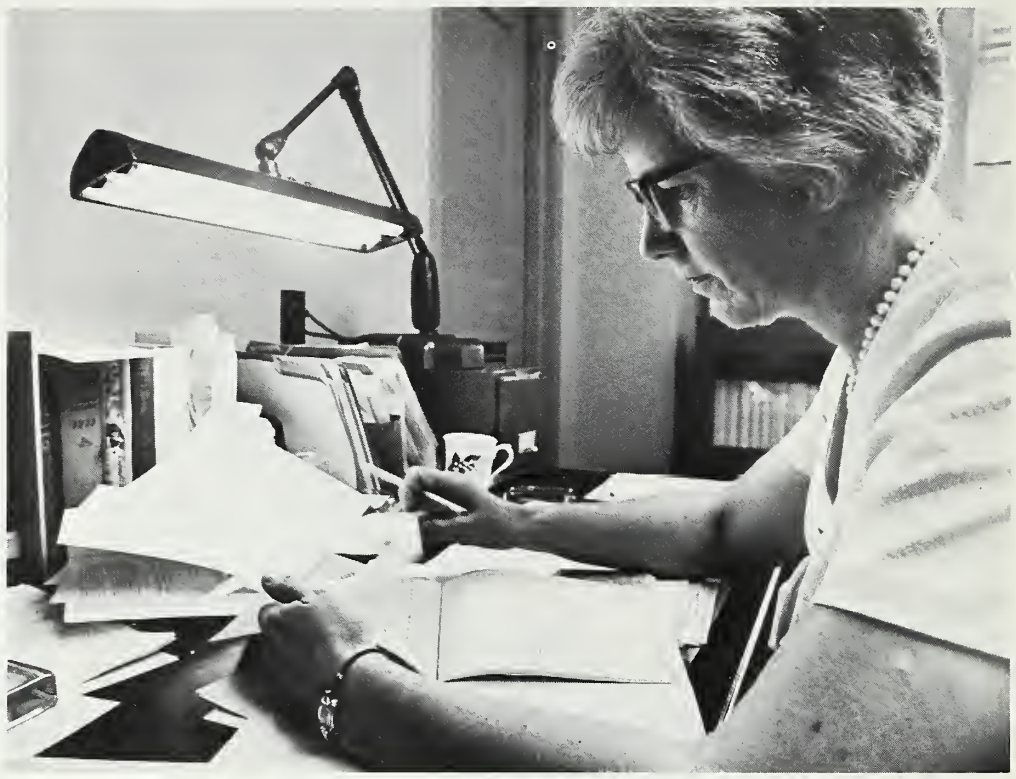
These rare compounds, aldobio- and aldotriouronic acids, will be chemically "fingerprinted" and used as reference models in research on starch-derived polysaccharides, says ARS sponsoring scientist, Dr. Allene R. Jeanes, at the Northern marketing and nutrition research laboratory, Peoria, Ill. The aim of this research is to produce new types of industrially useful polysaccharides from cereal grains to supplement or replace the more expensive plant gums which are now imported from abroad.

Polysaccharides are high molecular weight carbohydrates (macromolecules) which, on hydrolysis, yield simple sugars or simple sugars plus sugar derivatives such as uronic acids. New types of polysaccharides from cereal grains, called biopolymers, are produced by nonpathogenic micro-organisms which can transform the building unit of a cereal starch into numerous other sugar units and synthesize them into novel biopolymers.

Microbial polysaccharides include the dextrans and xanthan. A type of dextran discovered at the Peoria laboratory is used internationally as the source of a blood plasma substitute and as the source of various dextran derivatives employed widely in research and in the pharmaceutical and dairy industries. Xanthan, also discovered at the Peoria laboratory, is used in oil well drilling fluids and has been approved by the Food and Drug Administration as a thickening, stabilizing, emulsifying, and suspending agent in many foods.

Suitability of polysaccharides for specific uses depends upon such properties as solubility; viscous, rheological, and colloidal characteristics of the solutions; and stability of solution properties to shear, heat, salts, acids, and bases. The properties and structure of a biopolymer are determined by the chemical and physical makeup of the various part of the macromolecule and by the strain of micro-organism that produces it.

Isolating and fingerprinting the aldouronic acid components, which remain stable and intact after partial hydrolysis, is one way to gain information on the composition, structure, and behavior of the acidic biopolymers. These fingerprints also permit identification of unknown aldouronic acids when comparison samples are not available. □



Dr. Irwin analyzes data from reports on human nutrition requirements (0971X1138–4).

Review: Protein-amino acid research

A REVIEW of world literature shows the need for more research on nutritional requirements of the adolescent and elderly.

As described by ARS nutritionist Isabel Irwin, the objective of two studies involving man's requirements for protein and specific amino acids was to review, analyze, and summarize information by age groups and sex and to identify the areas where gaps exist. The studies will help establish priorities in planning and evaluating human nutrition research programs.

Chart under preparation shows state of knowledge of protein and amino acid requirements (0971X1138–18).

The protein and amino acid reviews, which were carried out under a cooperative agreement with Harvard University, revealed no reports of research during the past 50 years on the protein needs of healthy adolescents. Protein requirements of infants and young adults were studied the most. This means, Dr. Irwin said, that current protein recommendations for teenagers, commonly regarded as a nutritionally vulnerable group, are based on results of studies for adults and younger children. Amino acid requirements for growth have also not been studied except for infants.

Next to teenagers, studies of the protein and amino acid requirements of the elderly have been most neglected. Among the elderly, relatively few individuals have been studied, and the conclusions lack agreement.

Results of the two surveys also indicate the need for a better method of determining protein and amino acid requirements. Proteins, which are made up of amino acids, contain nitrogen in addition to the carbon, hydrogen, and oxygen found in fats, starches, and sugars. Because nitrogen is easier to measure than protein, scientists commonly study nitrogen balance when defining the needs of people and animals for protein. The difference between intake and outgo (nitrogen balance) indicates the amount of nitrogen retained by the body, thus providing what has been considered to be an accurate index of the amount of protein involved.

In her review, however, Dr. Irwin found frequent mention of apparent nitrogen retentions not accounted for by changes in body weight (more protein is needed during periods of growth) or body composition. This casts some doubt on the validity of nitrogen balance data not supported by other evidence.

The surveys also reveal poor correlation between estimates of protein and amino acid requirements. The estimated minimum requirement of high-quality protein is far greater than the amount necessary to supply the estimated amino acid requirements.

The two reviews show a need for experimental procedures that permit critical statistical evaluation of the data and for work with more subjects. The number of human subjects studied in controlled tests was surprisingly small.

Other reviews now in progress cover requirements for calcium and vitamins A, C, B_{12}, and folic acid. Preparations are being completed for reviews on iron, zinc, and other trace minerals. □

Soybeans aid air pollution studies

Soybean cotyledons may help scientists unravel some of the mystery surrounding the damage that air pollutants, particularly ozone, cause in plants.

ARS plant pathologist Robert K. Howell at Beltsville, Md. has found that, unlike leaves of other plants which are thin and extremely difficult to separate into their component parts for study, the cotyledons or first leaves of soybeans are fleshy and easily separated. The cotyledons also respond to ozone in a typical manner. This pollutant causes lesions on the upper surfaces of plant leaves, resulting in plant debilitation and reduced yields.

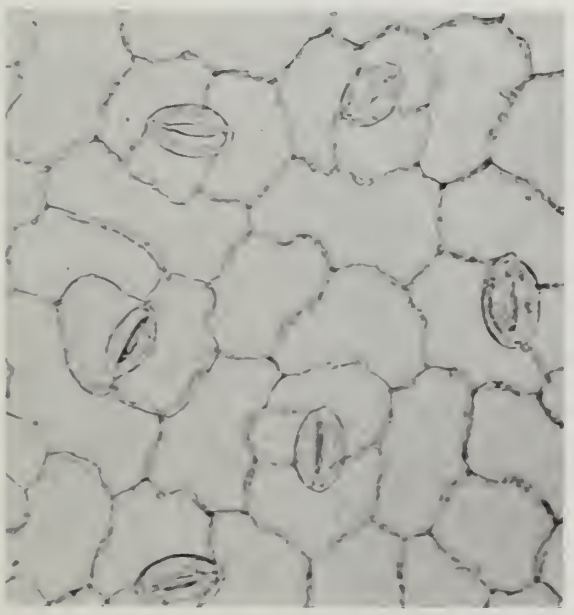
Further, cotyledons facilitate biochemical studies because they have a good supply of two distinct types of cells—upper cells called palisades and spongy cells on the lower surfaces.

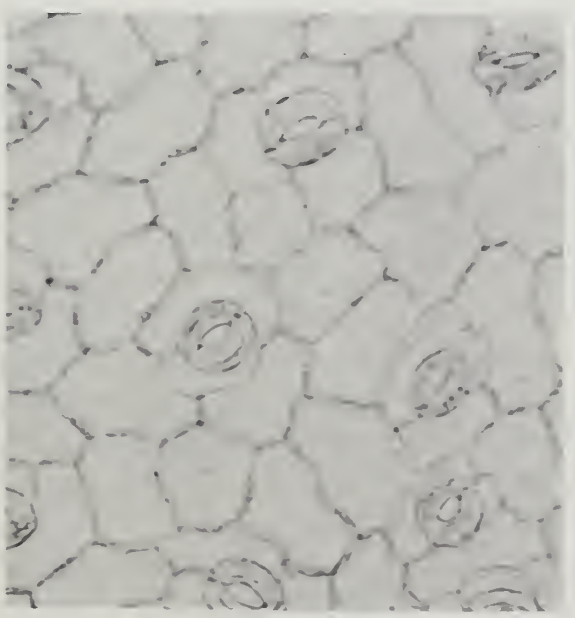
Dr. Howell learned that the cotyledons' epidermal layer could easily be stripped off by hand. He then could observe living stomata (leaf pores) directly under a light-microscope and see stomatal responses to ozone. Dr. Howell was also able to separate and study the differences in injured and noninjured palisade cells. By differential staining he could see clearly where the damage occurred, to what depth, and to what extent.

In his studies on the differential effects of ozone on five soybean varieties, Dr. Howell learned that not all varieties were injured to the same degree. Kent was the most severely injured, with Dare, Delmar, York, and Cutler incurring lesser injury in descending order. Injury to Kent, for example, was about five times more severe than that to Cutler.

The results of these and other studies will be useful in developing plant varieties tolerant of a broad spectrum of environmental stresses. And Dr. Howell says that soybean cotyledons offer an excellent medium for laboratory and classroom studies since the cotyledons are so accessible and easy to work with.

Left: *Ozone caused stomata (leaf pores) in soybean cotyledon tissue to close, thus possibly interfering with photosynthesis (PN–1990).* **Right:** *Open stomata—no ozone present (PN–1991).*





Rams pass chemical defleecing tests

Chemical defleecing, proved effective for quick, easy sheep shearing (AGR. RES., Oct. 1968, p. 8), has been demonstrated harmless to a ram's reproductive ability in dosages normally used.

ARS animal scientist Ivan L. Lindahl cooperated in two experiments in which various doses of the defleecing chemical, cyclophosphamide (CPA), were administered to rams. The experiments were conducted by E. Keith Inskeep and J. C. Herrington II of the West Virginia Agricultural Experiment Station, Morgantown.

In the first experiment, 0, 25, or 40 milligrams of CPA per kilogram of body weight were used. Semen volume, cell concentration, percent motile cells, percent abnormal cells, percent abnormal necks, and pH were measured.

In the second, the scientists administered 0, 30, and 40 mg CPA/kg and measured semen volume, cell concentration, motility and pH. Rams were bred to an average of nine ewes each to test CPA effect on fertility.

CPA at 25 and 30 mg/kg had no effect on any variable studied. The 40 mg level reduced sperm cell concentration and decreased percent motility in the sixth and seventh week after CPA treatment. Fertility was reduced in rams receiving 40 mg/kg when associated with reduced sperm cell concentration.

Lambs from matings to CPA-treated rams appeared to be quite normal.

Since 25 mg CPA/kg or less are normal dosages in defleecing, CPA used for defleecing rams does not affect reproduction, the scientists concluded.

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AGRISEARCH NOTES

AI cows yield more

Cows sired by bulls in artificial insemination (AI) studs are superior to cows sired by natural-service bulls (non-AI) in milk and butterfat production.

ARS animal scientists compared AI-sired cows with non-AI-sired cows within herds on a nationwide basis. Breeds studied were Ayrshire, Guernsey, Holstein, Jersey, and Brown Swiss on the Official Dairy Herd Improvement plan.

Calculations were made for four age-at-calving groups: 2-year-olds, 3- and 4-year-olds, 5-year-olds and up, and all ages. AI-sired cows were superior across all age groups, but superiority of AI-sired cows was generally greatest at younger ages. AI superiority gained for the years 1954 to 1968 but at a less rapid pace in the late 60's.

For 1968 calvings over all ages, AI-sired cows yielded from 117 to 387 pounds more milk, and from 3.4 to 12.3 pounds more fat than all non-AI-sired cows of the five breeds.

Quick test for wheat protein

A new biuret test for determining protein content in wheat cuts the time required to 5 minutes.

Chemist Robert M. Johnson and technician Carolee E. Craney, both of ARS at Beltsville, Md., earlier devised a biuret method for protein analysis in grain that correlated highly with the standard Kjeldahl test (AGR. RES., March 1971, p. 6). This method reduced test time from about 2 hours to 35 minutes. Industry needs, however, often demand even faster analysis.

To meet this demand, Dr. Johnson and ARS chemist Walter T. Greenaway substituted a 3-minute reaction step, using a reactor mill, for the 30-minute "shake and wait" step in the earlier biuret method. One gram each of powdered cupric carbonate and finely ground grain are added to the reactor mill along with 40 milliliters of alkali-alcohol solution.

After the reaction step, Dr. Johnson filters the mixture and reads the resulting filtrate's transmittance value in a colorimeter set at 550 nanometers using a 5-millimeter cell. Color intensity is directly proportional to protein concentration.

Meter readings of 32 samples representing five classes of wheat showed an excellent correlation with the Kjeldahl protein values.

Limes vary in ascorbic acid content

The concentration of ascorbic acid in Florida-grown Tahiti (Persian) limes is greatest in June and higher for small limes than large ones.

Citrus fruits have long been valued for their abundant source of scurvy-preventing ascorbic acid, vitamin C. Although Tahiti limes are harvested all year in Florida, information has been scarce concerning seasonal variation in ascorbic acid concentration, as well as variations according to fruit size and grove source.

To obtain this information, horticulturist Thurman T. Hatton, Jr., Orlando, Fla., and biological technician William F. Reeder, Miami, Fla., both of ARS, analyzed three sizes of Tahiti limes from three grove sources each month for 1 year.

Monthly average concentrations of ascorbic acid in limes from the combined three groves were significantly higher and varied less for small limes than large limes. The highest concentrations were 42.4, 34.6, and 31.0 milligrams per 100 milliliters of juice in limes 1⅞, 2⅛, and 2⅜ inches in diameter, respectively.

Ascorbic acid concentration was highest in June, lowest in March, and approximately the same for all of the other months. Limes picked during May and June had a greater ascorbic acid concentration range than those picked during the other 4 months, while those picked in February had the most narrow range.

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